Pak. J. *Agri. Sci., Vol.* 39(4), *2002*

PRODUCTION OF a-Amylase FROM BANANA PEEL BY *Bacillus subtilis*

R. Noreen, M. Asghar, M.J. Asad and O. Adedayo*
Department of Chemistry, University of Agriculture, Faisalabad.
*Dept.. of Chemical Engineering, University of Waterloo, Ontario, Canada.

We produced a-amylase from banana peel by *Bacillus subtilis* through solid state fermentation (SSF). Addition of yeast extract, corn steep liquor, sodium dodesyl sulphate (SDS) and Tween-80 enhanced the a-amylase production under preoptimized fermentation conditions. Maximum enzyme yield (10.25 U/mL) was observed at 35°C after 24 hours of SSF in growth medium containing 0.1% yeast extract, 1% corn steep liquor, 0.15% SOS and 0.1% Tween-80 at pH 7. The crude a-amylase produced under optimum conditions was characterized by studying the effect of pH and temperature on its activity. Activity of a-amylase was maximum (12.18 U/mL) at 60°C and pH 6 which was stable for 3 hours under these conditions.
Key words: a -amylase, solid state fermentation, Banana peel, *Bacillus subtilis,* optimization, stability.

INTRODUCTION

Amylases, of bacterial origin, are used in numerous starch processing industries, such as baking brewing and in the production of sugar syrups (Asghar *et al.,* 2000). It is also extensively used in paper, food, pharmaceutical, detergent and textile industries (Nigam and Sing, 1995; Haq *et al*., 2002). However, its production is expensive and a search for the most cost effective fermentation strategy for its production is desired (Anonymous, 1989). The solid state fermentation (SSF) of an inexpensive substrate and use of surfactants has the potential to increase the enzyme yield and reduce production costs (Hanes and Stedt, 1998). Microbial a-amylases could be potentially useful in the pharmaceutical and fine chemical industries if enzymes with suitable properties could be prepared with the advent of new frontiers in biotechnology (pandey *et al., 2000).*

Major waste products of banana industry are the pseudostem, stalks (Peduncles), banana fruit peel and over ripened whole banana (Goewert and Nicholas, 1980).

Commercial application, may be found for the banana peel wax, possibly for use in shoe or furniture polishes, however, it has been demonstrated that banana pulp or peel could be used as a fermentable substrate for the production of single cell protein and hydrolytic enzymes (Chung and Meyers; 1979). The present research was planned to investigate into the possibility of using banana peel waste through SSF for cost effective production of a-amylase by *Bacillus subtilis* which will also dispose off this abundant waste by a pollution free technology.

MATERIALS AND METHODS

Substrate: Fresh banana peel obtained from a fruit shop in the shopping center of University of Agriculture, Faisalabad was used as substrate for a-amylase production. The substrate was chopped into small pieces of uniform particle size (40 mm) with knife. Fresh peel was used in each experiment.

Fermentative organism

Pure culture of *Bacillus subtilis* obtained from NIBGE, Faisalabad was raised on nutrient agar stants maintenance medium at pH 7 and 35°C (Krishna and Chanderrasekaran, 1996).

Inoculum Preparation

For inoculum preparation, the inoculumn medium was prepared and its pH was adjusted to 7 with 1MHCl/1 MNaOH. It was sterilized in an autoclave fer 15 minutes at 121°C. A loopful culture of *Bacillus subtilis,* raised on nutrient agar slants, was transferred aseptically in laminor air flow into the inoculum medium. The liquid medium was then incubated for 24 hours at 37°C on a shaker (150rpm) for microbial growth to get at 10^7_10^8 spores/m l and the homogenous spore suspension was used as inoculum.

Fermentation Process

Banana peel (50 g) containing 84% moisture was taken in each conical flask and pH was adjusted at 7 with MHCl/MNaOH. The basal medium contained (g/100 mL): peptone, 0.2; $MgSO_4.7H_20$, 0.02; $CaCl_2.2H_20$, 0.04 and KH_2P0_4, 0.1 (Kokab, 2002). The flasks were autoclaved for 15 min. at 121°C. Spore inocula (5 ml) containing 10^7_10^8 spores/ml were then added into each flask with the help of a 10 mL sterilized glass pipette and shaken for uniform distribution of inoculum. For a-amylase production the flasks were incubated at pH 7 and 35°C temperature, without shaking for 24 hours. After every 12 hours the flaks were gently shaken for mixing.

To all the fermented biomass samples 100 mL of 0.02 M sodium phosphate buffer of pH 6.9 was added and the flasks were shaken for 1 hour at 150 rpm. After separation of residue by filtration, the filtrates were centrifuged at 1000 rpm for 10 minutes to remove the spores of the organisms. The supematants were

carefully collected and used as crude enzyme solution.

Optimization of culture conditions

Yeast extract

Five different concentrations of yeast extract (0.001, 0.05, 0.1%, 0.15 and 0.2%) were added into the growth medium of chopped banana peel along with pre-optimized ingredients.

Effect of corn steep liquor

Five different concentrations of corn steep liquor viz., 0.5, 1, 1.5, 2 and 2.5% were used in duplicate along with optimum concentration of yeast extract (0.1%).

Effect of sodium dodocyl sulphate (SOS)

To investigate the effect of sodium dodcyl sulphate (SOS) as surfactant on SSF, five different concentrations (0.05%, 0.1%, 0.15%, 0.2% and 0.25%) of SOS were used in duplicate in the presence of optimum concentrations of yeast extract (0.1%) and corn steep liquor (1.0%).

Effect of Tween 80

To enhance the production of a-amylase in SSF, another surfactant, tween-80 was added into the banana peel medium at five different concentrations (0.05, 0.1, 0.15, 0.2, 0.25 and 0.25%) with pre-optimized culture conditions.

Enzyme Assay

Activity of a-amylase was measured by the spectrophotometric method described by Bernfeld (1955), in an assay mixture containing enzyme extract, starch as substrate and ONS as coupling reagent.. One unit of a-amylase activity was defined as the amount of enzyme which released 1 IJm of maltose by 1 mL original enzyme solution in one minute.

RESULTS AND DISCUSSION

Optimization of process parameters

Yeast Extract

Medium supplemented with 0.1% (w/w) yeast extract yielded maximum (8.14 U/mL) a-amylase activity. Further addition of yeast extract caused a gradual decreased in enzyme production (Table 1). The results of our study support Krishna and Chandrasekaran (1996) who also have reported that *Bacillus subtilis* produced maximum a-amylase in the optimum growth medium of banana stalk containing 0.1% yeast extract and a decrease, thereafter.

Corn Steep Liquor

With the addition of corn steep liquor with the SSF medium of banana peel, the production of a-amylase increased steadily and was maximum (9.17 U/mL) at 1.0% (w/w) level.. Further addition of corn steep liquor (2.0%) caused a decreased in enzyme yield (Table 2). Addition of corn steep liquor upto 1.0% level in culture medium supplied additional nutrients and sugars as energy source. Further addition of corn steep liquor decrease the production of a-amylase which may be due to catabolic repression (Silva and Yang, 1998).

Effect of SOS

Addition of SOS enhanced the a-amylase production. Optimum enzyme activity was observed with 0.15% (w/w) SOS. Further addition of SOS (0.2 and 0.25) decreased a enzyme production (Fig. 1). Goes and Shapparad (1999) used SOS to enhance the production of a-amylase by *Bacillus subtilis* and observed that SOS at 0.05% (w/w) concentration provided the maximum enzyme activity. when compared with control..

Table 1. Activity of a-amylase produced by *Bacillus subtilis* with varying concentrations of yeast extract under optimum conditions*

Yeast Extract (%)	a-amylase activity (U/mL)		
	A	B	Mean
Control	5.10	5.11	5.10
0.001	6.59	6.57	6.58
0.05	7.58	7.52	7.55
0.1	8.38	8,43	8,41
0.15	7.15	7.10	7.12
0.2	6.28	6.27	6.27

*Banana peel 50g; inoculum, 5 ml; $MgS0_4$, 0.02%; $CaCl_2$, 0.04%; KH_2P0_4, 0.1%; pH 7; Temperature 35°C (Kokab, 2002).

Effect of Tween-80

Addition of Tween-80 increased the production of a-amylase. Its activity was maximum (10.25 U/mL) with 0.15% (w/w) concentration of Tween-80 (Fig. 1). Arnesen *et al.* (1998) have reported a 2.7 fold increase in a-amylase activity by Tween-80 as compared to control.. Our results also support Goes and Shappered (1999) who reported that non ionic synthetic surfactant tween-80 enhanced the production of a-amylase by *Bacillus subtilis* and 0.1% of this surfactant provided the maximum enzyme activity.

Fig.1. Activity of a-amylase produced by *Bacillus subtilis* with varying concentrations of SOS and Tween-80

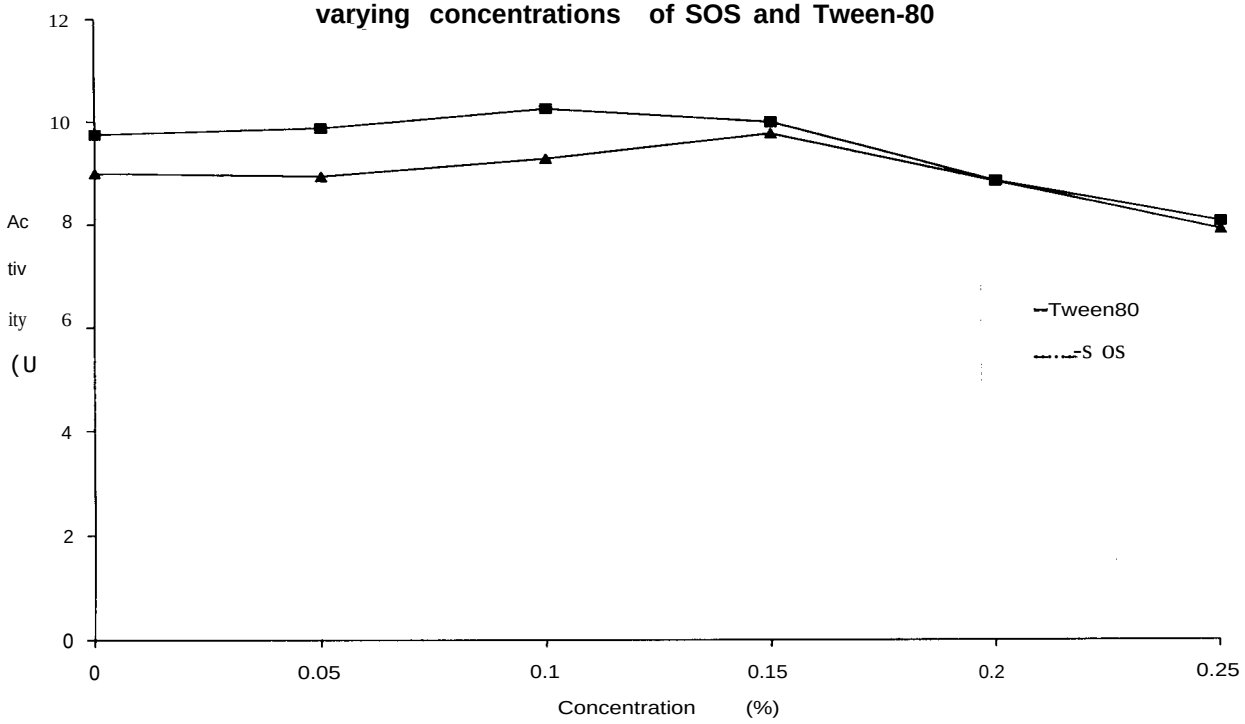

Fig. 2 Effect of pH on crude a-amylase activity

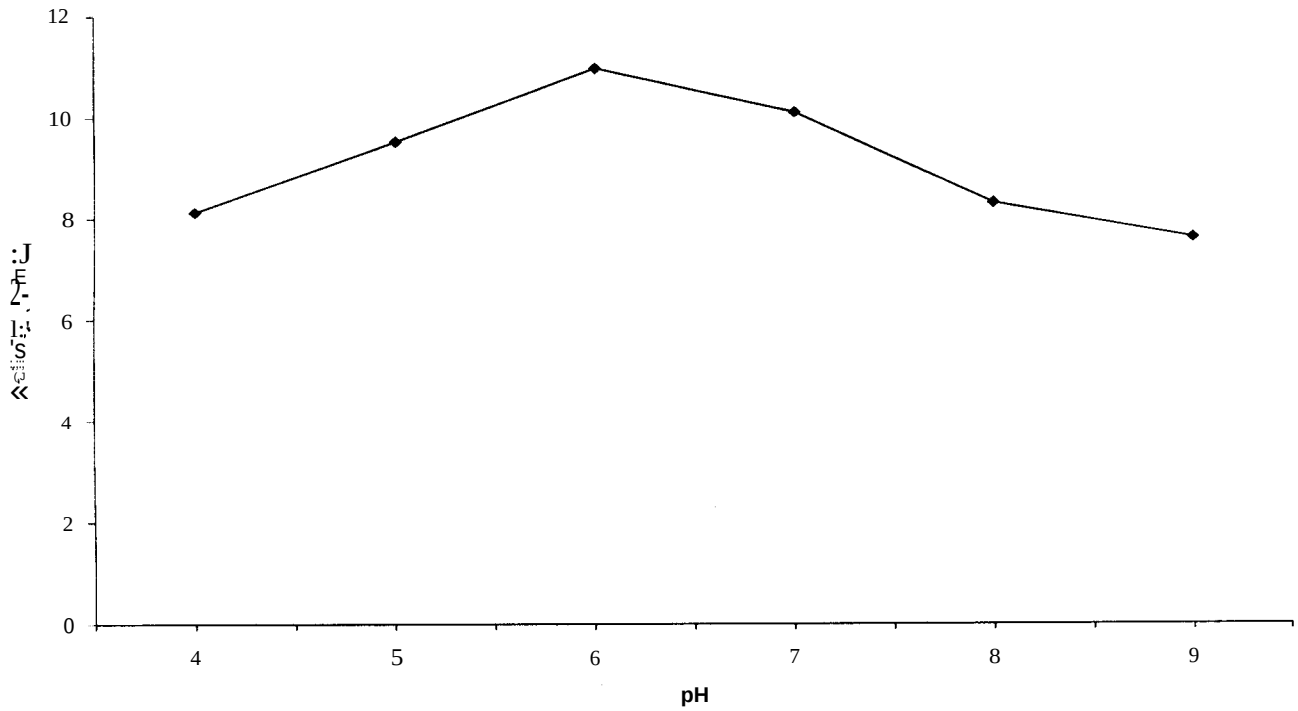

Fig.3 Effect of temperature on crude a-amylase activity

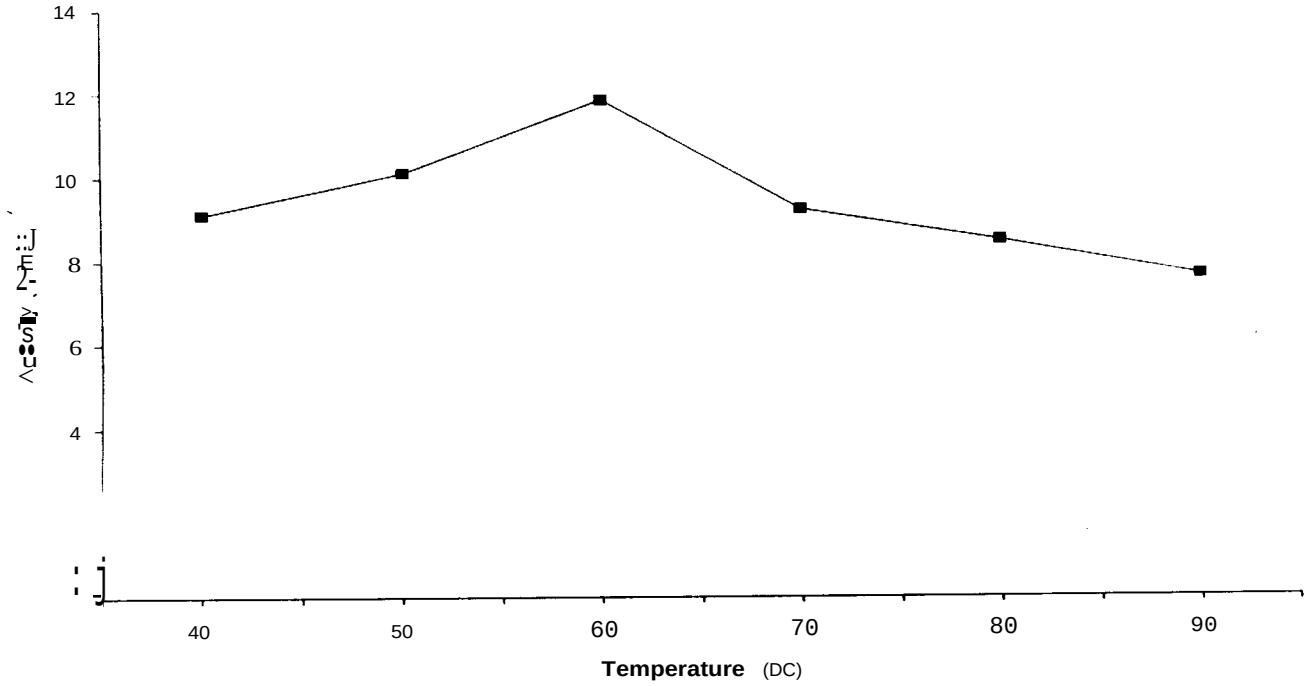

Fig.4 Stability of a-am ylase produced by *Bacillus subtilis* at optim um pH and tem perature

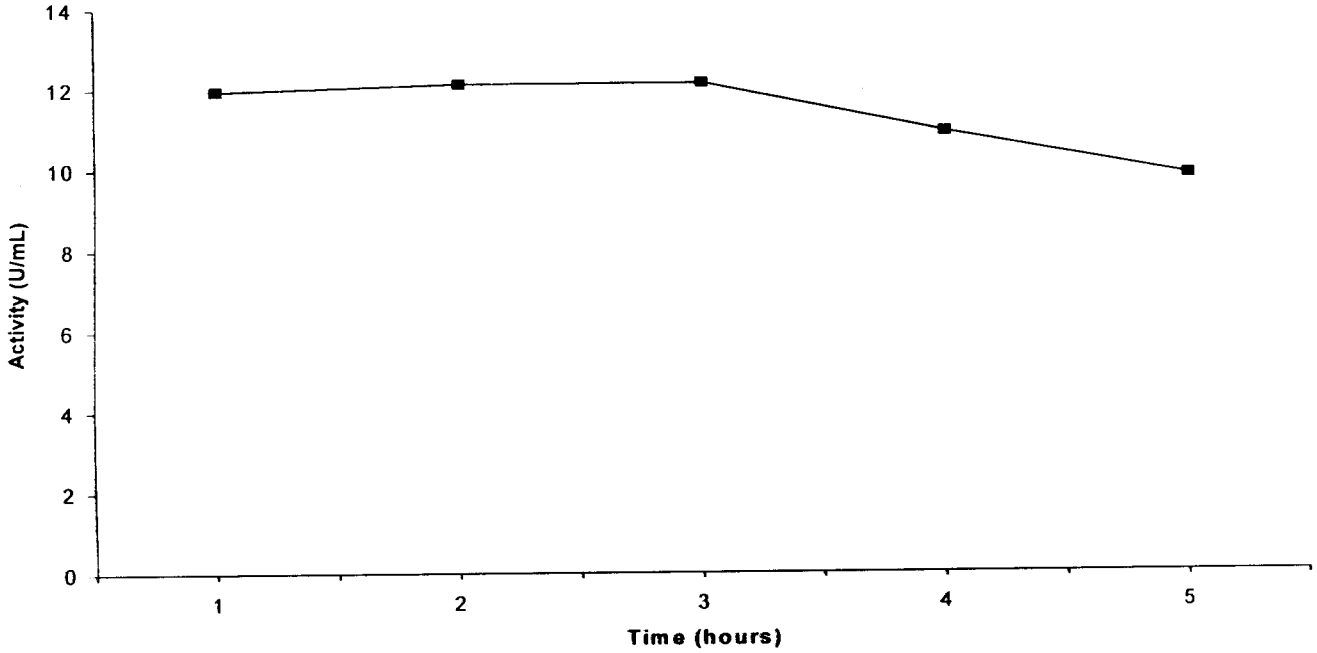

Table 2. Activity of a-amylase produced by *Bacillus subtilis* in a medium containing 0.1% yeast extract and varying concentrations of corn steep liquor

Corn steep liquor (%)	a-amylase activity (U/mL)		
	A	B	Mean
Control	8.39	8.40	8.39
0.5	8.48	8.24	8.45
1.0	9.21	9.13	9.17
1.5	7.81	7.79	7.8
2.0	6.86	6.63	6.65
2.5	8.10	5.12	5.0

Kinetic Studies of a-amylase

Each enzyme has its characteristic optimum pH and temperature for its maximum activity. To determine the optimum pH, temperature and stability of a-amylase under optimum conditions, the enzyme was assayed under varying assay conditions.

Effect of pH

The pH of medium influences the production of a-amylase. The enzyme activity was maximum at pH 6. On either side of pH optimum, the activity of enzyme was decreased (Fig. 2). Hamilton *et al.* (1999) also reported maximum activity of a-amylase produced by *Bacillus sp.* at pH 6.0. Yang and Wang (1999) reported optimum pH between 6.0 and 7.0 for a-amylases produced by *Streptomyces rimosus* TM-55 with both the submerged and solid state cultivation methods.

Effect of temperature

Crude enzyme was assayed at different temperatures and activity of enzyme was increased by an initial increase in temperature which was maximum (11.85 U/mL) at 60°C. Activity of a-amylase decreased on either side of the optimum temperature (Fig. 3). Mamo and Gassesse (1999) reported 55°C optimum temperature for maximum activity of a-amylase produced by *Bacillus sp.* Castro *et al.* (1999) noted maximum activity of a-amylase produced by *Bacillus licheniformis* MIR-61 at 50 to 67°C. In our results a-amylase showed maximum activity at 60°C and this minor difference may be due to different microbial source of the enzyme.

Stability under optimum conditions

The enzyme was incubated under optimum conditions (pH, 6, 60°C) for different time periods. The enzyme was stable for 3 hours started loosing it's activity (Fig. 4). Lin *et al.* (1998) reported that optimum temperature and pH for activity of a-amylase were 70°C and 9 respectively. Jana and Pati (1997) observed the maximum pH and temperature for a-amylase activity produced by *Bacillus sp.* MD-124 as pH 6 and 90°C, respectively and enzyme was stable for 24 hours under these conditions. Yang and Wang (1999) also observed that enzyme activities produced by *Streptomyces rimosus* TM-55 in SSF were more stable with pH and temperature changes than those produced in submerged cultivations.

CONCLUSION

It was concluded that banana peel can be effectively used in solid state fermentation for a-amylase production by *Bacillus subtilis.* The enzyme thus produced showed optimum pH and thermostability characteristics which indicated its SUitability for industrial applications.

REFERENCES

Anonymous. 1989. Consus repair of Pakistan. Production Consus Organization, Statistics Division, Govt. of Pakistan, Islamabad.

Arnesen, S., S.H. Eriksen, J. Olsen and B. Jensen. 1998. Increased production of a-amylase from *Thermoamyces lannginosus* by the addition of Tween 80. Enz. Microb. Technol. 23(3-4):249-252.

Asghar, M., M.J. Asad and M. Arshad. 2000. a-amylase from *Aspergillus niger* in waste bread medium. Proc. 2nd Int. Symp., New Technol. for Environ. Monit. and Agro-Appli. October 18-20, Tekirdag, Turkey. PP. 561-564.

Bernfald, P. 1955. Amylases a and P Methods in enzymology, Vol. 1. Academic Press, New York. PP. 149.

Castro, G.R., M.D. Baigori and F. Sinerzi. 1999. Studies on a-amylase production by *Bacillus-licheniformis* Mir-61. Acta Biotechnologica, 19(3):263-272.

Chunq, S.L. and S.P. Meyers. 1979. Bioproteins from banana wastes. Developments in Industrial Microbiology. 20:723-32.

Goes, A.P. and J.D. Shappard. 1999. Effect of surfactant on alpha-amylase production in a solid state fermentation process. J. Chemical Technol. Biotechnol. 74(7):709-712.

Goewert, RR and H.J. Nicholas. 1980. Banana peel sugars as a source of food stuff for animals or humans. Nutrition Reports Int, 22:207-12.

Hamilton, L.M., CT Kelly and W.M. Fogarty. 1999. Production and properties of the raw starch digesting alpha-amylase of *Bacillus sp.* Ind. 435. Process Biochemistry. 35(1-2):27-31.

Hanes, B. and S. Stedt.. 1988. Milling and Banking Technology. John Wiley and Sons USA PP:415-470.

Haq, I., H. Ashraf, S. Rani and MA Qadeer. 2002. Biosynthesis of alpha amylase by chemically treated mutant of *Bacillus subtilis* GCBU-20. Pak. J. Biol. Sci., 2:73-75.

Jana, M. and B. Patio 1997. Thermostable solid tolerant alpha-amylase from *Bacillus sp.* MD-124. J. Basic Microbial 37(5):323-326.

Kokab, S. 2002. Utilization of Banana Coverings for (x,-amylase Production by *Bacillus subti/is.* M.Sc. Thesis, Deptt. Chem. Univ. Agri., Faisalabad. Pak.

Krishna, C. and M. Chandrasekaran. 1996. Banana waste as substrate for alpha-amylase production by Bacillus subtitles (CBTK-106) under solid-state fermentation. Appl.. Microbial.. Biotechnol.. 46(2):106-111.

Lin, L.L., C.C. Chyan and W.H. Hsu. 1998. Production and properties of a raw-starch degrading amylase from the thermophillic and alkalophillic *Bacillus sp.* TS-23, J. Biotech. Appl. Biochem. 28:61-68.

Mamo, G., BA Gashe and A. Gessesso. 1999. A highly thermostable amylase from a newly isolated thermophilic *Bacillus sp.* Wanll. J. Appl.. Microbial.. 86(4):557-560.

Nigam, P. and D. Singh. 1995. Enzymes and microbial system involved in starch processing enzyme. Microb. Technol.. 17:770-778.

Pandey, A., P. Nigem, D. Singh and R Mohan. 2000. Review advances in microbial amylases. Biotech. and Applied Biochem. 31(2):135-152.

Silva, E.M. and ST Yang. 1998. Production of amylases from rice straw by solid state fermentation in a gass solid spited bed bioreactor. Biotechnol.. Progress. 14(4):580-587.

Yang, S.S. and J.Y. Wang. 1999. Protease and amylase production of strepotmyces rimoasus in submerged and solid state cultivations. Botanical Bulletin of Academia Sinica.. 40(4):259-265.